

The acid-base condition in vegetation, litter and humus: IV. The strength of the acidoids and the relation to nitrogen.

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The apparent strength of litter and humus acidoids.

It has been shown in parts I and II of this series of papers that if the acidoid content of the samples is plotted against the pH_u values obtained in a 1:10 suspension in N. KCl solution most of the points lie close to a curve the equation of which is

$$(\text{H}^+) = 0.067 [\text{A}]$$

where $[\text{A}]$ expresses equivalents of acidoids per liter.

Nearly all of the mature samples of litter, fresh as well as decomposed, and all samples of humus investigated showed only minor deviations from this linear relationship. Greater deviations were found for all the samples of vegetation and for those of the litter which were especially rich in nitrogen such as the leaves of alder and the decomposed leaves of ash, all of which were characterized by higher pH_u values (cf. fig. 8 and 11).

Eliminating these major deviations we have plotted the data for the remaining material including 94 samples as shown in fig. 23. The figure gives the pH_u values in water (crosses) as well as in N. KCl solution (circles) both plotted against the acidoid content in the 1:10 suspension, expressed in terms of standard concentration of equivalents per liter.

We note that the pH_u values in water are much more scattered than the pH_u values in KCl. The suspension effect in water is apparently a very erratic factor due, probably, to variations in the Donnan potential. In the presence of KCl this potential is suppressed and the H ions, which are displaced by the K ions move out into the intermicellar solution.

But although the relationship to the acidoid content is not so

definite in the case of the pH_u in water as it is in the case of the pH_u in KCl it is nevertheless evident that such a relationship does exist and that it is a different relationship from that of the pH_u in KCl solution. This is evident from the fact that the difference between the pH_u in water and the pH_u in KCl, i.e., the exchange acidity, increases with increasing acidoid content (cf. table 24).

Table 24. *The average acidoid concentration (in 1:10 suspension) within each increment of 25 milliequivalents acidoids per 100 grams and the corresponding pH_u in water and in N. KCl solution. Also the corresponding apparent dissociation constants k_w and k_s , the ratio $(H_s^+)/[A]$ and the exchange acidity ($w-s$) of 94 samples of litter and humus. (Cf. fig. 23.)*

Acidoids M.e./100 g.	No of samples	Acidoid conc. in 1:10 susp. [A]	pH_u in:		(H_w^+) $\times 10^{-3}$	(H_s^+) $\times 10^{-2}$	k_w $\times 10^{-5}$	k_s $\times 10^{-3}$	$\frac{(H_s^+)}{[A]}$ $= c$	$w-s$
			water w	N.KCl s						
0—25	4	0.021	3.32	2.76	0.479	0.174	1.11	0.16	0.084	0.56
25—50	3	0.039	3.29	2.62	0.513	0.240	0.67	0.16	0.061	0.67
50—75	19	0.062	3.16	2.39	0.692	0.407	0.78	0.29	0.066	0.77
75—100	37	0.089	3.11	2.26	0.776	0.549	0.68	0.36	0.062	0.85
100—125	14	0.113	3.05	2.10	0.891	0.704	0.71	0.60	0.071	0.95
125—150	11	0.136	2.99	2.02	1.020	0.955	0.76	0.72	0.070	0.97
150—175	4	0.159	3.05	1.87	0.891	1.350	0.50	1.26	0.085	1.18
253 & 261	2	0.257	2.98	1.74	1.050	1.820	0.43	1.39	0.071	1.24
General average for k_w and c :							0.72		0.067	

In order to simplify a closer study of the relationships we calculated the average pH and the average acidoid concentration for each increment of 25 milliequivalents acidoids per 100 gram. The eight average values thus obtained are given in table 24.

From the general mass law equation

$$\frac{(H^+)(S^-)}{(HS)} = \frac{(H^+)^2}{(HS)} = K$$

we then calculated the apparent dissociation constants k_w and k_s of the acidoids in water and in N. KCl solution, respectively, by substituting the concentration of the total acidoid $[A]$ minus the hydrogen ion activities (H_w^+) and (H_s^+) for the activity of the undissociated acid (HS) in the above equation thus

$$\frac{(H_w^+)^2}{[A] - (H_w^+)} = k_w \quad (1)$$

and

$$\frac{(H_s^+)^2}{[A] - (H_s^+)} = k_s \quad (2)$$

The results are shown in table 24.

We note that, except for the first group of 4 (all straw) and the last group of 2 samples (both Mølper humus) the average k_w values are remarkably constant in spite of the great variations among the 94 individual samples. The apparent strength of the acidoids in pure water is therefore approximately the same for all the materials included in the figure independent of their acidoid content. Their apparent strength is of the same order of magnitude as for some of the common organic acids (for acetic acid $K = 1.82 \times 10^{-5}$ at 18°C). Since (H_w^+) is very small we can put $[A] - (H_w^+) = [A]$ in equation (1) and get then the equation

$$(H_w^+) = \sqrt{k_w[A]} \quad (3)$$

according to which the H ion activity of the materials suspended in pure water (1:10) is (approximately) proportional to the square root of the acidoid concentration analogous to a weak acid (cf. upper curve fig. 23).

The k_s values show no such constancy but increase from 0.16 to 1.39×10^{-3} . The apparent strength of the acidoids in KCl solution increases, therefore, with the acidoid content of the samples. The relationship between the H ion activity and the acidoid concentration is expressed by the ratio

$$\frac{(H_s^+)}{[A]} = c$$

which is remarkably constant with an individual average value of 0.067.

We thus obtain the linear relationship

$$(H_s^+) = 0.067 [A]$$

from which we find that the H ion activity of *equal weights* (cf. below) of the materials in KCl solution is directly proportional to the acidoid content (cf. lower curve in fig. 23). The percentage dissociation (or displacement of H ions) appears thus, in N. KCl solution, to be constant and amount to an average of 6.7 percent.

The acidities of the samples increase, therefore, faster in the salt solution than in water with an increase in the acidoid content. The result of this is that the exchange acidity is greater the greater the acidoid content. This is shown in the last column in table 24 and is expressed by the divergence of the curves in fig. 23 which show

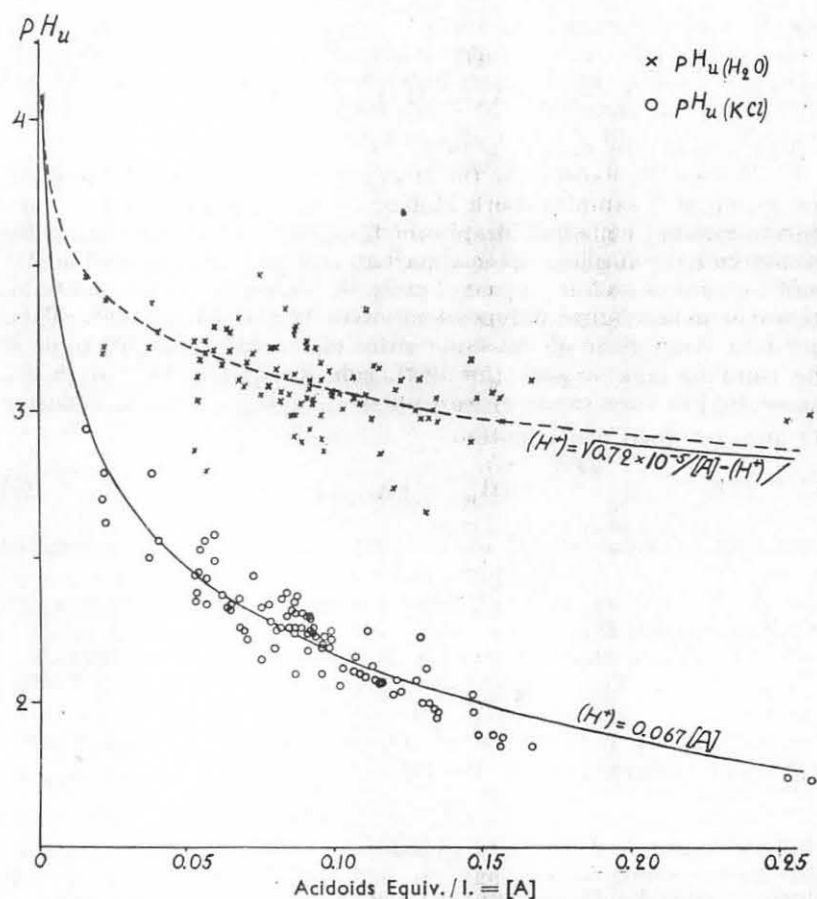


Fig. 23. The pH_u in water (\times) and in $N.KCl$ solution (O) of equal weights (1 : 10) of 94 samples of litter and humus in relation to the acidoid content. The curves are calculated on the basis of the average values of the constants k_w and c in table 24.

the theoretical relationships as based on the average values in table 24 for the two constants k_w and c . We note that these curves intersect when extrapolated to a pH about 4. If the relationship between the two series of pH_u values holds for materials having a very small acidoid content then there should be no exchange acidity for such materials. Yellow pea flour, extracted with ether and electro dialysed yielded in one case a very slight exchange acidity and in another case none at all, the pH_u values in water being 3.79 and 3.69 and in KCl 3.74 and 3.69 in the first and second case respectively. This

and other experiments on amphoteric behavior will be discussed in one of the two following parts of this series.

It might in this connection be recalled that MATTSON and HOU (1937), who had obtained exchange alkalinity at low pH in the case of podzol humus, failed to get this result in the case of a rendzina humus no matter how low the pH nor what salt solution was used. We can now understand why. The rendzina humus like the Mölner has a very high acidoid content and yields a strong exchange acidity which cannot be entirely suppressed by the addition of acid.

If the curves in fig. 23 represent the actual trend in the relationship then there seems to be only one workable theory. This can be stated as follows:

Litter and humus acidoids appear to be stronger in a salt solution the greater the acidoid content because the proportion of organic basoids (basic nitrogen) is lower the higher the acidoid content, that is, the acidoid formation takes place at a higher rate than the basoid formation and the isoelectric point (I.E.P.) is, therefore, lower the higher the acidoid content. When KCl is added to an unsaturated material having a low acidoid content — and a relatively high basoid content — a great deal of the HCl formed by the cation exchange is neutralized by the basoids and we get a relatively high pH_u . If the material has a high acidoid content — and a relatively low basoid content — a smaller proportion of the HCl will be neutralized and we get a relatively low pH_u .

That the differing capacities of the various forms of litter and humus to bind base cannot be due to the presence of *varying amounts* of a *definite acidoid* or ampholytoid was indicated by the observations of BORATYŃSKI and MATTSON (1939) who found the same differences in the capacities of the NaOH-extracted and Ca-precipitated humus as in the untreated raw materials. We shall, however, now present a still more conclusive evidence of differences in the amphoteric complex of the different materials.

If the amphoteric complex present in the different materials differed only with respect to the amount present and not with respect to the strength or proportion of the acidoid and basoid groups then the pH_u ought to be the same for all materials in suspensions containing equivalent quantities of acidoids. Preliminary experiments with the original and the decomposed leaves of beech, birch and maple showed that, when the decomposed samples — which in the 1:10 suspension had a considerably lower pH_u than the original samples — were so diluted that the acidoid concentration was the same as that in the suspension of the original samples the decomposed samples continued to be decidedly more acid than the original.

We then proceeded to make a systematic study of the dilution effect on our most acidic material, the Mölner humus, which permitted an increase in concentration up to 6 grams in 10 cc N. KCl solution.

The sample used in this work consisted of a mixture of equal parts of the Mölner I and II the acidoid contents of which were 253 and 261 m.e./100 g. respectively. The results are shown in table 25.

Table 25. *The pH_u in N. KCl solution of the Mölner humus at various concentrations of the suspension and the apparent dissociation constant of the acidoids.*

Grams in 10 cc	M.e. Acidoid A	Conc. A. Equiv./l. [A]	pH_u	(H^+) ($\times 10^{-2}$)	$k_s = \frac{(H^+)^2}{[A] - (H^+)}$ ($\times 10^{-3}$)
$\frac{1}{64}$	0.04	0.004	3.05	0.089	0.25
$\frac{1}{32}$	0.08	0.008	2.76	0.174	0.48
$\frac{1}{16}$	0.161	0.0161	2.53	0.295	0.66
$\frac{1}{8}$	0.321	0.0321	2.30	0.501	0.93
$\frac{1}{4}$	0.643	0.0643	2.09	0.813	1.18
$\frac{1}{2}$	1.285	0.1285	1.91	1.23	1.30
1	2.57	0.257	1.74	1.82	1.39
2	5.14	0.514	1.59	2.57	1.35
4	10.28	1.028	1.47	3.39	1.16
6	15.42	1.542	1.37	4.27	1.22
Average k_s for $\frac{1}{4}$ to 6 g. in 10 cc:					1.26

A comparison of tables 24 and 25 shows that the H ion activity in KCl solution does not decrease as fast with a decrease in the concentration of acidoids when we dilute one and the same sample of humus, e.g., the Mölner, as when we take the same weight of different samples possessing varying acidoid content. The relationship is shown graphically in fig. 24. Between the concentrations 0.064 to 1.54 equivalents acidoids per liter the Mölner yields apparent dissociation constants which are fairly close to an average value of 1.26×10^{-3} . An acid whose dissociation constant equals this value should give the lower, broken curve shown in fig. 24 when the pH of the solution is plotted against the concentration of the acid. It will be noted that above an acidoid concentration of about N. 0.05 the Mölner dilution curve coincides almost exactly with the theoretical curve.

A dilution experiment in pure water was performed with a sample of the Annerstad highmoor peat (cf. table 21). The results yielded a fair approach to a constant according to equation (1) or in this case, where (H^+) was very small, according to equation (3).

The dissociation of the humus acidoids apparently proceeds in salt solutions as well as in pure water according to the law of weak acids.

The linear relationship obtained in KCl solution when equal weights of different samples are taken must depend on different acidoid/

basoid ratios, i.e., on different I.E.P.s. In pure water the acidoid groups, being by far the strongest, dominate the reaction of the suspension. But in the salt solution where free acid is formed, by exchange, which combines with the basoid groups the reaction is modified according to the proportion of the latter. Why the relationship is linear is another question which might be difficult to answer. It seems to point to the existence of a definite law governing the

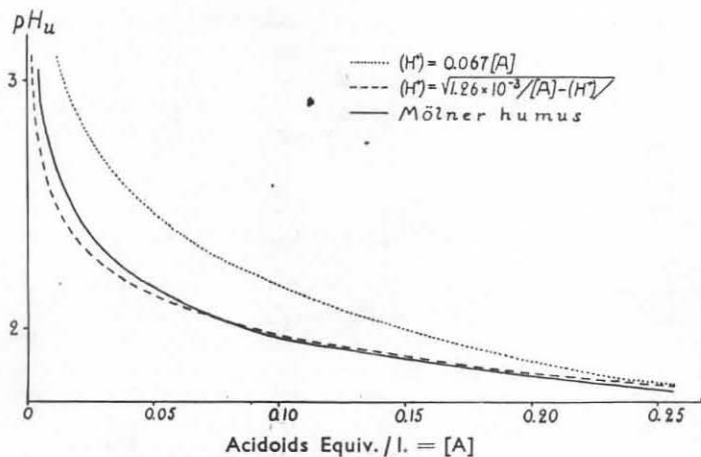


Fig. 24. The pH_u in N.KCl solution of the Mölnner humus in relation to the acidoid content when the ratio humus: solution is varied. The broken line curve is calculated on the basis of the average constant k_s in table 25. The dotted curve is the same as the lower curve in fig. 23.

amphoteric nature of decaying organic matter. We shall return to this question in connection with the nitrogen content.

The Mölnner dilution curve intersects the curve of the 94 equal weight samples (1:10 = 100 g./l.) at a point which corresponds to the acidoid content of the Mölnner sample, i.e., at 0.257 equivalents per 100 gram. If each of the 94 samples give the same type of dilution curve as the Mölnner then each such curve would intersect the equal weight curve at a point corresponding to the acidoid content of the sample.

It is of interest to compare the Mölnner dilution curve with a corresponding curve of an acid possessing a definite dissociation constant. The constant of tartaric acid is 0.97×10^{-3} at 25°C . We found a pH of 1.76 in a N. 0.5 solution of a sample of commercial tartaric acid at room temperature. This is close to that found in a 1:10 suspension of the Mölnner humus (= 1.74).

Fig. 25 gives a graphic representation of the result obtained by

dilution of the tartaric acid solution together with that of the Mölnér humus.

Now if the linear relationship $(H^+) = 0.067 [A]$ found when equal weights of the 94 samples are taken depends on an increase in the proportion of basoids (basic N or sesquioxides or both) as the acidoid content decreases it is of interest to determine the effective quantities of base involved. This was done experimentally by titrating the various dilutions of the Mölnér humus with NaOH

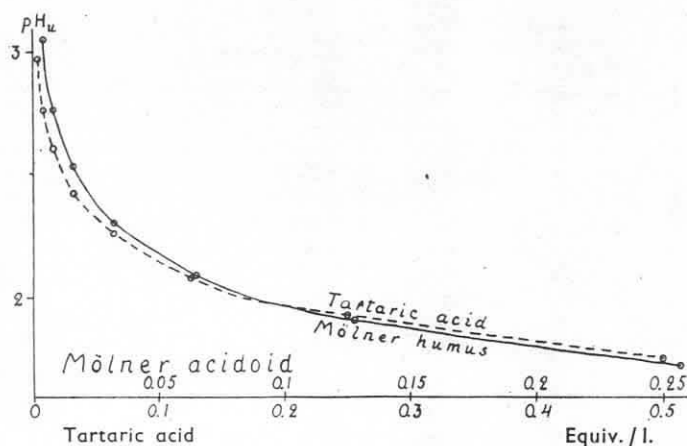


Fig. 25. The Mölnér humus dilution curve (same as in fig. 24) compared to a dilution curve for tartaric acid.

to the corresponding pH on the equal weight curve of the 94 samples, that is, we determined the quantities of base required to lower the acidoid activity in the various dilutions from the square root relationship to the linear. The following (interpolated) amounts of base were thus found to be required:

Humus g.....	1.0	0.5	0.25	0.125	0.062
M.e. acidoid (A)	2.57	1.28	0.64	0.32	0.16
base required (B)	0.0	0.10	0.075	0.055	0.033
B/A $\times 100 = \%$	0.0	7.8	11.7	17.2	20.6

If the acidoids in the 94 samples of litter and humus are all of the same strength then the figures in the above tabulation would mean that the acidoids are increasingly «neutralized» with basoids up to a saturation of 20.6 percent as their acidoid content decreases from 2.57 to 0.16 milliequivalents per gram or from the content of Mölnér humus to that of wheat straw.

It should be added that the above percentage increase in base saturation was found to be considerably greater when dilutions of the tartaric acid solution were similarly titrated or when the theoretical amounts of base required were calculated from the »buffer equation» for the case of an acid whose dissociation constant equals 1.26×10^{-3} . A monobasic acid having this constant ($pK = 2.90$) is, however, half saturated at pH 2.90 and 99 percent saturated at pH 4.90 whereas the humus acidoids bind a large part of the base, which expresses the acidoid content, at higher pH. The concentration of acidoids which dissociate at low pH is therefore, in reality, considerably smaller than the concentration determined by titration to pH 7, as here done. The apparent dissociation constants of the strongest acidoid groups is, therefore, greater than the values given in our tables.

The function of nitrogen.

The nine fresh samples of litter (F_0 samples) which were used in our study of decomposition, including wheat straw, the needles of pine and the leaves of beech, birch, ash, alder, elm, oak and maple showed, with the exception of alder, a fairly close adherence to the linear relationship between the H ion activity of the electro dialysed samples and their acidoid content (cf. fig. 26).

The unique position of alder was believed to be related to its high nitrogen content. In agreement with the findings of HESSELMAN

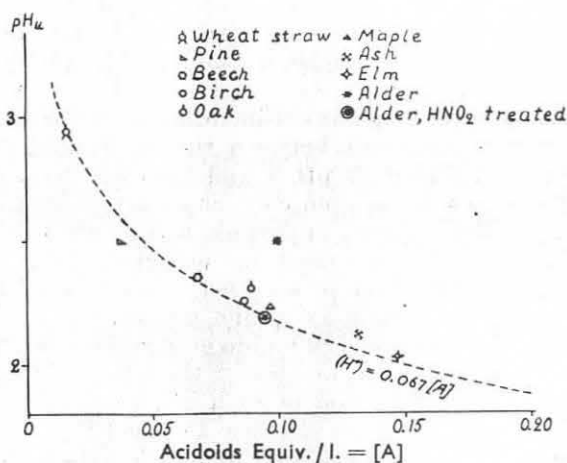


Fig. 26. The pH_u in N.KCl solution (1:10) in relation to the acidoid content of eight samples of fresh tree litter (F_0 samples) and wheat straw. Alder is shown before and after treatment with nitrous acid. The curve is the same as the lower curve in fig. 23.

(1925) we had found a strong evolution of NH_3 during the decomposition of the alder leaves. Ordinarily no NH_3 is formed in the decomposition of organic matter containing a high proportion of carbohydrates, the N being assumed to be tied up in the form of microbial protein (WAKSMAN 1938). Hesselman found NH_3 evolved only in the cases where the N content of the material exceeded about 2 percent.

If the high percentage of nitrogen (3.96 %) is responsible for the abnormally high pH_u value of the alder leaves it should be possible to prove this by splitting of the NH_2 groups by treatment with nitrous acid according to the Van Slyke method. Ten grams of the powdered leaves were therefore suspended in an acetic acid solution and digested with 2 grams of NaNO_2 until the evolution of gas ceased. The material was then thoroughly washed and electro dialysed. The results are shown in table 26.

Table 26. *The nitrogen and acidoid contents and the pH_u of alder leave-litter before and after treatment with nitrous acid.*

Material	N % ¹	Acidoid M.e./100 g. ¹	pH_u in:	
			water	N. KCl
Before treatment	3.96	100.0	3.04	2.50
After *	4.66	95.0	2.79	2.19

The nitrous acid treatment reduced the pH_u in KCl solution from 2.50 to 2.19. The latter value comes very close to the linear relationship averaged by the 94 samples of litter and humus (cf. encircled asterisk in fig. 26).

That the acidoid content was not increased by the treatment would indicate that the inner salt between the CO_2H and NH_2 groups is completely hydrolysed at pH 7 and that the NH_2 group does, therefore, not affect the acidoid content as determined by titration to this pH. It is, of course, impossible to say what other changes than deamination the treatment has brought about. The increase in the nitrogen content may be ascribed to the formation of nitroso compounds with the secondary amino groups.

If the removal of the primary amino groups increases the strength of plant and humus acidoids, i.e., lowers the I.E.P. of the amphoteric complex then an introduction of such groups should produce the opposite effect and cause an increase in the pH_u of the material. It is known that when humus is treated with NH_3 a part enters in a nonexchangeable form (FEUSTEL & BYERS 1933). How does the so bound NH_3 affect the strength of the acidoids?

¹ On the basis of dry weight of electro dialysed material.

In order to determine this we digested 20 grams of each of the four Häggbygget podzol samples V, F₁, F₂ and H (cf. Part III) with 200 cc concentrated ammonia in covered Erlenmeyer flasks for one week after which the samples were evaporated to dryness on the steambath, powdered and electrodyalysed. The results are shown in table 27.

Table 27. *The increase in nonexchangeable nitrogen in ammoniated V, F and H samples of the Häggbygget podzol and its effect on the acidoid strength.*

Material	M.e./100 g.: *		N %	pH _u in:		Exchange acidity w-s
	acids ¹	acidoids		water w	N. KCl s	

A. Untreated:						
V	32.5	49.5	1.04	3.27	2.50	0.77
F ₁	19.5	62.5	1.10	3.16	2.37	.79
F ₂	13.0	79.0	1.61	3.22	2.28	.94
H	12.0	91.0	1.69	3.22	2.24	.98

B. NH ₃ -treated:						
V	58.0	43.0	2.20	3.57	2.96	.61
F ₁	49.0	59.0	2.53	3.41	2.92	.49
F ₂	47.5	77.0	3.23	3.56	2.85	.71
H	56.0	92.0	3.47	3.59	2.68	.91

C. Difference (B-A):						
V	25.5	-6.5	1.16	0.30	0.46	-.16
F ₁	29.5	-3.5	1.43	0.25	0.55	-.30
F ₂	34.5	-2.0	1.62	0.34	0.57	-.23
H	44.0	1.0	1.78	0.37	0.44	-.07

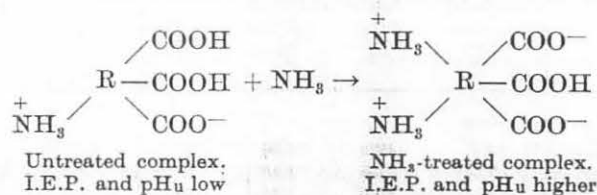
We note that the NH₃-treatment has considerably increased the content of diffusible acids whereas the acidoid content is not greatly affected. As in the NaOH-treated samples (cf. Part III) a part of the acidoids might have passed through the parchment and thus appear as «acids». Of special interest is the fact that the NH₃-treated samples have fixed from 1.16 to 1.78 percent ammonia nitrogen and that the pH_u values have at the same time increased. This increase is greater in the KCl solution than in water. The exchange acidity has therefore been reduced.

The nonexchangeable ammonia has obviously strengthened the basoid groups and thus elevated the I.E.P. of the complex. The formation of an inner salt causes the apparent strength of the acidoids to be decreased. Whether this likewise applies to the apparent acidoid content, i.e., the capacity to bind base at pH 7, cannot now

* ¹ Anode solution.

be decided. This depends entirely on the strength of the new basoid groups: on the hydrolysis of their inner salts. An addition of 1.16 to 1.78 percent N is equivalent to 83 to 127 m.e. NH_3 per 100 grams. It might therefore be considered very probable that the capacity to bind base at pH 7 has been reduced even if only a part of the fixed ammonia enters as basic nitrogen. The somewhat reduced acidoid content in three of the samples might be ascribed to this cause; especially with reference to the fact that the NaOH-treated samples showed a great increase in acidoid content.

Assuming the ammonia to enter the complex as primary amino groups we might formulate the reaction by the following scheme:



The titration of the free COOH group takes place according to its own dissociation constant whereas the capacity of the inner salt (the zwitter ion) to bind base takes place according to the hydrolysis constant of the amino group ($= K_{\text{water}}/K_{\text{base}}$) since it is only the condition of this group which is affected by the added base:

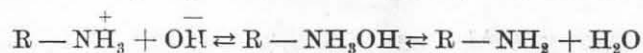


Table 27 brings out another relationship which, if it should prove to be general, is of fundamental importance. It will be seen that the percentage increase (B—A) in nitrogen is very closely proportional to the acidoid content. The following ratios are obtained:

Sample	V	F ₁	F ₂	H
Acidoids m.e./100 g. (A) ..	49.5	62.5	79.0	91.0
NH_3 fixed " " (B) ..	82.9	102.1	115.7	127.1
A/B	0.60	0.61	0.68	0.71

The significance of this proportionality between the chemical fixation of ammonia and the acidoid content is heightened by the fact that the nitrogen originally present in the samples is itself proportional to the acidoid content.

If we plot the nitrogen content in terms of milliequivalents of NH_3 against the acidoid content before and after the treatment of the samples with ammonia we get the picture shown in fig. 27.

As pointed out in the preceding paper of this series the same relationship was found to exist in the series of fresh litter (F_0 samples) included in fig. 26, again with the exception of alder. This relationship, which is surprisingly close, is shown in fig. 28.

Other F and H samples thus far analysed with respect to their nitrogen content in the electro dialysed condition are listed in table 28 together with their acidoid content. Even this heterogeneous collection shows evidence of a high degree of correlation, which is clearly brought out in fig. 29.

The only marked deviation is that of the Tiohaga podzol F_1 sample. This material consists exclusively of spruce needles the green (V) samples of which is relatively high in nitrogen (1.81 %) and very low in acidoids (26 m.e.). The F_1 sample might be considered to be of the same type as the alder leaves or, shall we say, in an «unstable» acidoid/basoid condition. This expresses itself in an abnormally high pH_u in KCl solution (= 2.79. Cf. first lone circle from the left side above the unbroken curve in fig. 23) and likewise a

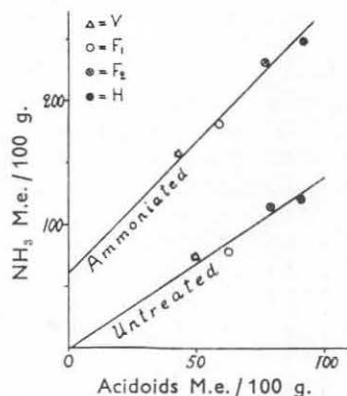


Fig. 27. The relationship between the acidoid content and the content of nitrogen (expressed as NH_3) in four electro dialysed samples of the Häggbyget podzol before and after being ammoniated.

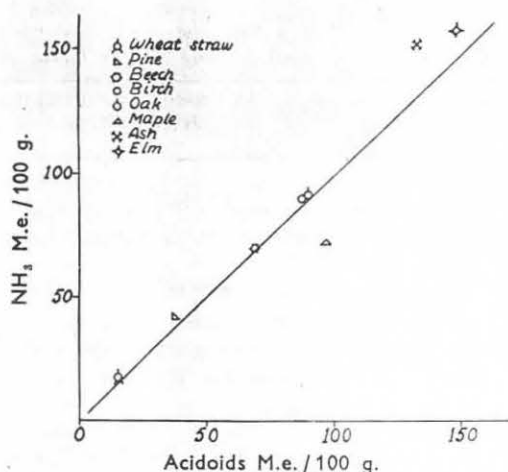


Fig. 28. The relationship between the acidoid and nondialyseable nitrogen content of the samples described in fig. 26 (except alder).

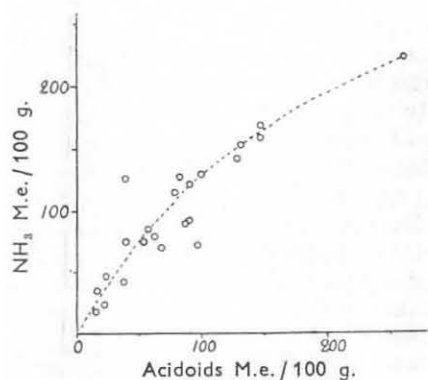


Fig. 29. The relationship between the acidoid and nondialyseable nitrogen content in the materials described in table 28, in fig. 27 (untreated samples) and in fig. 28.

the apparent strength of the acidoids with a decrease in acidoid content and (2) a decrease in the exchange acidity in the same direction. The presence of only one point at the upper end of the curve, however, makes any conclusion uncertain. It is conceivable that the sesquioxide basoids, assuming the percentage of these to be about the same in all samples, are responsible for the behavior. The nature of the relationship between acidoids and nitrogen is now being further investigated.

Table 28. The percentage nitrogen and the acidoid content of various samples of electrolysed straw and humus on the basis of dry matter.

Straw	N %	Acidoids M.e./100 g.	Humus	N %	Acidoids M.e./100 g.
Rye	0.49	16.0	Tiohaga ¹ F ₁	1.77	38.6
Oat	0.32	21.7	" F ₂	1.78	82.6
Barley	0.65	22.7	" F ₃	1.81	99.5
Clover	1.05	53.4	" H ₁	1.98	129.4
Pea	1.19	57.2	Mölnar ² II	3.15	261.0
			" III	2.36	147.0

Discussion.

The exception is often more instructive than the rule. The exception encountered in the case of the alder leaves has established one of the dominating factors in the observed relationship between the

¹ Cf. Part II.

² Cf. Part III.

low exchange acidity (= 0.58. Cf. table 17 Part II). The more »normal» acidoid/nitrogen ratio of the F₂ sample collected in a 2 cm lower layer might be taken as an indication of the rapidity with which the litter adjusts itself to the »stable condition», in this case not by losing nitrogen but by greatly increasing its acidoid content.

The position of the points in fig. 29 seems to indicate that the proportion of nitrogen is greater at lower than at higher acidoid content. Such is also the relationship demanded to account for the observed behavior, viz. (1) a decrease in

pH_u in KCl solution and the acidoid content. This factor is the content of basoid nitrogen (= nondiffusible basic nitrogen). The aforementioned relationship postulates, therefore, another relationship namely the relationship between the acidoid content and the content of basoid nitrogen. From the data here presented (cf. fig. 27—29) it seems very evident that chemical and biochemical reactions tend to establish a certain relationship in the proportions of acidoids and nitrogen.

This observation will help to clarify the much discussed C/N ratio which by many has been assumed to tend to be constant for humus. If the nitrogen bears a definite relationship to the acidoids the C/N ratio must vary with the acidoid content. Having found that the acidoid content depends on the base status and aeration (cf. Part III) we can easily account for the inconstancies in the C/N ratio and its reported trend in the various soil types (wider ratios in the humid than in the arid regions, wider in the surface than in the subsoil, wider in anaerobic peat than in aerobic mor or mull, etc.).

Waksman ascribes the humus nitrogen to the formation of microbial protoplasm and bases this conclusion on the observation that «cellulose decomposes only in proportion to the available nitrogen» (loc. cit. p. 118). Why the microbial protoplasm should be so much more stable than the protoplasm of the cells of the higher plants is difficult to understand.

On the basis of our findings it seems more probable that the amphoteric humus complex is an extra-cellular product; a digestive residue of microbial activity, a stabilized and, with respect to energy levels as well as to structure, undifferentiated product of plant materials. Substances which are unstable have been removed whereas such which increase the stability of the complex have been added by chemical as well as by biochemical action. Acidoids are formed and a certain proportion of basic nitrogen tends to be maintained, by loss if in excess and by fixation, possibly in the form of ammonia, if deficient. An amphoteric complex would be expected to be more stable, at least electrokinetically, than a pure acidoid or a pure basoid. In this sense the formation of humus might be looked upon as a process of isoelectric precipitation or «weathering».

We wish to emphasize the fact that the observed relationship between acidoids and nitrogen applies to the fresh litter as well as to humus. The litter has not been acted upon by microorganisms, its structure has not been destroyed, the soluble constituents have not been leached out and the carbohydrates have not fermented. The C/N ratio in the litter is, therefore, in general high. All this tends to obscure any chemical kinship between the amphoteric complex of litter and humus such as here revealed.

In the case of the living plant material (V-samples) we do not find the same definite relationships with respect to amphoteric behavior.

This is to be expected for any such relationship would rob nature of the power of infinite variation which we constantly observe. The chemistry of the living cell is governed by the laws of the dynamics of living matter. By utilizing energy from external sources the reactions run «uphill», the machinery is wound up, the «batteries» are charged and the materials differentiated.

The litter, on the other hand, occupies a place close to humus inasmuch as the chemistry of both is determined by the laws of the dynamics of dead matter. Before the falling of the leaves the charging of the «batteries» has ceased and the current of polarization has begun to run down. No wonder then if the changes in the amphoteric properties tend to go in one direction in all forms of litter. How the chemical properties then gradually change from the fresh litter to the structureless dark humus has been shown. We find all gradations so that, chemically, it is impossible to draw a line and say that this is a litter complex and that is a humus complex. From $F_0, F_1 \dots F_n$ to $H_1, H_2 \dots H_n$ the series is continuous.

We thus arrive at the conclusion that the amphoteric humus complex is directly derived from the litter (including microbial litter) and represents a «digestive residue» produced by enzymatic action but modified also by purely chemical action. The «nucleus» of the humus complex is, according to this view, not synthesized in the process of humification but in the living plant. The specific chemical properties of humus originate at the death of the plant material.

The major chemical changes taking place during the process of humification consist, under aerobic conditions, in an increase in the acidoid content and in a simultaneous fixation of basoid nitrogen (probably as ammonia) which bears a definite relationship to the acidoid content. But the acidoid formation seems to take place at a higher rate than the formation of basoids because the apparent strength of the acidoids is greater and the I.E.P. of the complex is lower the higher the acidoid content.

These properties are ultimately determined by the soil conditions (base status and aeration) rather than by the composition of the original litter.

Summary.

Observations:

1. The H ion activity of the electro dialysed F and H samples in 1:10 suspensions in N. KCl solution is directly proportional to the acidoid content. Exceptions to this rule are those abnormally high in nitrogen.
2. Dilutions of one and the same sample yield H ion activities

which are proportional to the square root of the concentration of the undissociated acidoid similar to weak acids.

3. The apparent strength of the acidoids and the exchange acidity of the electro-dialysed samples increase with an increase in the acidoid content.

4. The nitrogen content bears a definite relationship to the acidoid content except in some F samples which are abnormally high in nitrogen. The acidoids increase, however, faster than the basoids so that the isoelectric point is lowered with an increase in the acidoid content.

5. The chemical fixation of ammonia in a nonexchangeable form is likewise proportional to the acidoid content.

6. The apparent strength of the acidoids and the exchange acidity in samples originally high in nitrogen or those ammoniated are both relatively low. The splitting off of nitrogen by the nitrite treatment increases the apparent strength of the acidoids and the exchange acidity.

Conclusion:

1. The amphoteric complex in litter and humus is of the same nature, the latter being directly derived from the former by a continuous series of changes. These changes consist primarily of an increase in the acidoid and nitrogen (basoid) content which certain chemical forces tend to keep in a definite relationship to each other.

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